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Title
Multiple Components Mapping of Live Tissue by Phasor Analysis of Fluorescence Lifetime Imaging

Permalink
https://escholarship.org/uc/item/6jm029bt

Journal
Biophysical Journal, 98(3)

ISSN
0006-3495

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Publication Date
2010

DOI
10.1016/j.bpj.2009.12.1155

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interactions within the physiological context. Here we use the phasor approach to fluorescence lifetime imaging to analyze complex decays in a live tissue. The tissues used were seminiferous tubules from the testes of wild type mice or mice expressing GFP from an Oct4 transgene. Lifetime images were acquired in the time domain and analytically transformed in the phasor representation. By examination of the clustering of the phasors we identified different molecular components: auto fluorescence, GFP, collagen and retinol. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of the fluorophores and emphasizes the contribution of the species to the signal. To better identify specific tissue components we also used spectral imaging and second harmonic generation microscopy. Linear combinations in the same pixel of molecular species were recognized and their relative fraction was calculated and mapped. The analysis of the fluorescence decay with higher harmonics of the phasor plot separates different molecular components that have the same location in the phasor plot at one harmonic but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique and straightforward method for interpreting complex decays in terms of molecular features by identifying fluorophores and obtaining functional maps of their relative concentration. This method has the potential to become a non invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues. Work supported by NIH-P41 P41-RRO3155 and P50-GM076516, NIH RO1 HD49488, NIH PO1 HD47675, CIRM RC1-00110 PD.

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